NanoFabTx™ Microfluidic - nano, device kit

Protocol for Catalog No. 911593

Introduction

NanoFabTx™ nanoformulation reagent and device kits enable users to encapsulate a wide variety of therapeutic drug molecules for targeted or extended drug delivery without the need for lengthy trial-and-error optimization. ***NanoFabTx™*** kits provide an easy-to-use toolkit for encapsulating a huge variety of therapeutics in nanoparticles, microparticles, or liposomes. The resulting particles are biocompatible and biodegradable and can be further modified to target specific tissues or to ensure slow and sustained drug release. Drug encapsulated particles synthesized with ***NanoFabTx™*** kits are suitable for biomedical research applications such as oncology, immuno-oncology, gene delivery, and vaccine delivery.

NanoFabTx™ reagent kits (sold separately) include carefully optimized polymer or lipid blends, a compatible stabilizer and step-by-step instructions for synthesizing drug-encapsulated formulations. Protocols for two different particle synthesis methods are included in each kit; one protocol uses the nanoprecipitation method to prepare drug-encapsulated nanoparticles in standard laboratory glassware and the second protocol uses microfluidics-based synthesis employing either a commercial microfluidics platform or syringe pump.

***NanoFabTx™* Microfluidic - nano device kits** are designed to work together with ***NanoFabTx™* -Nano** reagent kit and can be used to create drug-encapsulated polymeric nanoparticles or liposomes with narrow size dispersity and high batch-to-batch consistency. The device kit includes a comprehensive protocol, pre-assembled microfluidic chip manifold, tubing, and required accessories and is compatible with either the Dolomite microfluidics system or a standard syringe pump (compatible microfluidics system or syringe pump sold separately). Under most conditions, pressurized microfluidics pumps can yield improved reproducibility compared to the use of syringe pumps.

This kit was developed and tested in partnership with Dolomite Microfluidics. For compatible pumps and microfluidic systems, please visit: https://www.dolomite-microfluidics.com/products/nanofabtx-hardware-solutions/

Disclaimer

***NanoFabTx™* Microfluidic - nano device kit** is for research use only; not suitable for human use. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Specifications

Storage	Store *NanoFabTx™* Microfluidic - nano device kit in a clean dry place at room temperature.
Stability	Refer to the expiration date on the batch-specific Certificate of Analysis.

Materials

Materials supplied

Each *NanoFabTx™* Microfluidic - nano kit is preassembled with the following components:

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Replacement Part Catalog Number	Description	Part Label in Figure 1	Quantity
911917	Microfluidic Chip (Micromixing Chip)	Chip	1
	Tubing: Pre-cut lengths of tubing (250 µm FEP) (preassembled)		
	Junction: T-Junction	T1, T2	2
	Connector: Linear connector 4 way	Linear input/output connector	2
917230	Interface: Chip Interface H	H-Interface	1
	In-line Valve: In-line Valves	V1, V2, V3	3
	Plug: Plug 1.6 mm	Blocking plug	4
916609	End Fittings and Ferrules: End Fittings and Ferrules for 1.6 mm Tubing	L1, L2, and L3	
	Luer lock: Female to female Luer lock (for syringe pumps)	Female to female luer lock	3
917877	Filters: In-line filter		3
917494	Extra tubing roll (250 µm FEP-10m)		1

Materials required, but not supplied

Catalog Number	Description
	NanoFabTx™ reagent kits required for microfluidics protocol; (Further information available at www.sigmaaldrich.com/nanofabtx)
V7130	Glass scintillation vials (20 ml capacity)
SLFH025	Syringe filters 0.45µm (for filtering non aqueous solvents like, acetonitrile and DMSO)
	Solvents: Dimethyl Sulfoxide (DMSO) (Cat.No. 276855) is recommended for priming and cleaning for use with polymer or lipid reagents. Alternatively, Ethanol (Cat.No. 459836) can be used for priming and cleaning for use with lipid reagents.

Materials required for use with the Dolomite Microfluidics system, but not supplied

Catalog Number	Description
	Pressurized pump system (protocol requires two or three pumps) (e.g. Dolomite Mitos P-Pump). Further information for compatible Dolomite Microfluidic pumps and microfluidic systems can be found at https://www.dolomite-microfluidics.com/products/nanofabtx-hardware-solutions/.
	Dolomite flow sensors (protocol requires two flow sensors). Further information for compatible Dolomite Microfluidic flow sensors https://www.dolomite-microfluidics.com/products/nanofabtx-hardware-solutions/.

Materials required for use with syringe pumps, but not supplied

Catalog Number	Description
	Syringe pumps (protocol requires two or three pumps) (e.g. Harvard Apparatus – PHD Ultra pumps)
	Syringes compatible with syringe pumps required (recommended with Hamilton® GASTIGHT® syringes, Cat. No. 26211-U).

Before you start: Important tips for optimal results

Reduce blockages with proper cleaning. Always clean the microfluidics system after synthesis of each batch of drug-encapsulated nanoparticles. Insufficient cleaning can result in blockages in the micromixing microfluidics chip and tubing. A well-maintained microfluidics chip can be used multiple times if cleaned and stored properly.

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Prime the tubing and chip. Prime the tubing and the micromixing microfluidics chip before starting nanoparticle or liposome synthesis. Priming purges gases from the fluid pathways, conditions the chip surface with the stabilizers, and serves as a check of chemical compatibility for all wetted parts of the system. In addition, priming reduces or prevents precipitation of reagents inside the system in the case of backflow, jetting, or chaotic mixing. Precipitation of reagents can irreversibly block the microfluidics chip.

Using syringe pumps: When using syringe pumps, gradually increase flow rate in a stepwise fashion to the desired flow rate. Ensure valves are open in the flow path, since back pressure can build up and cause leaks.

Procedure

The **microfluidics-based method** can be used to synthesize nanoparticles or liposomes with narrow size distribution, enhanced control over each stage of particle fabrication, greater particle yields, ease of scalability, and excellent reproducibility compared to traditional synthesis techniques. This microfluidics protocol is for use with a Dolomite Microfluidics system (**Figure 1**) or syringe pumps (**Figure 2**). Under most conditions, microfluidics pumps can yield improved reproducibility compared to use of syringe pumps.

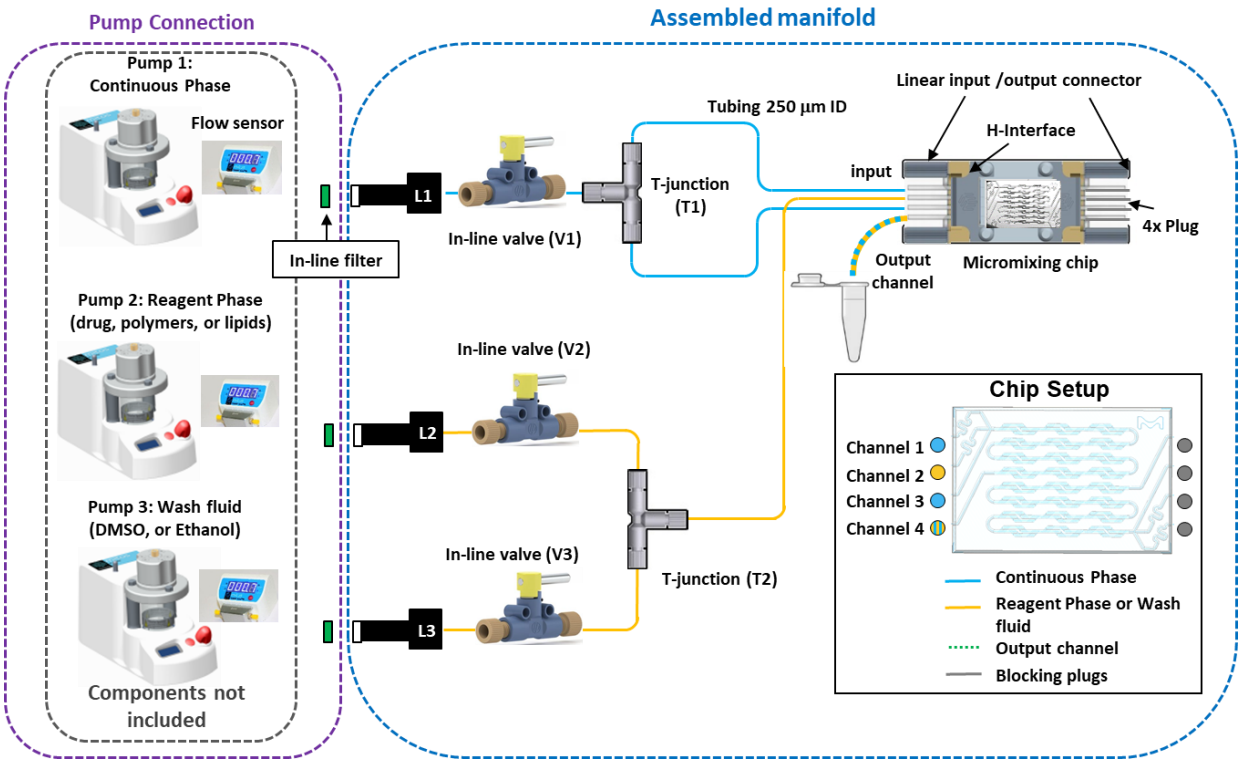

Figure 1: *Schematic of the microfluidics setup for the Dolomite Microfluidics system. The manifold is supplied preassembled. The microfluidics chip is packaged separately. In-line filters are supplied for connection to microfluidic pumps.*

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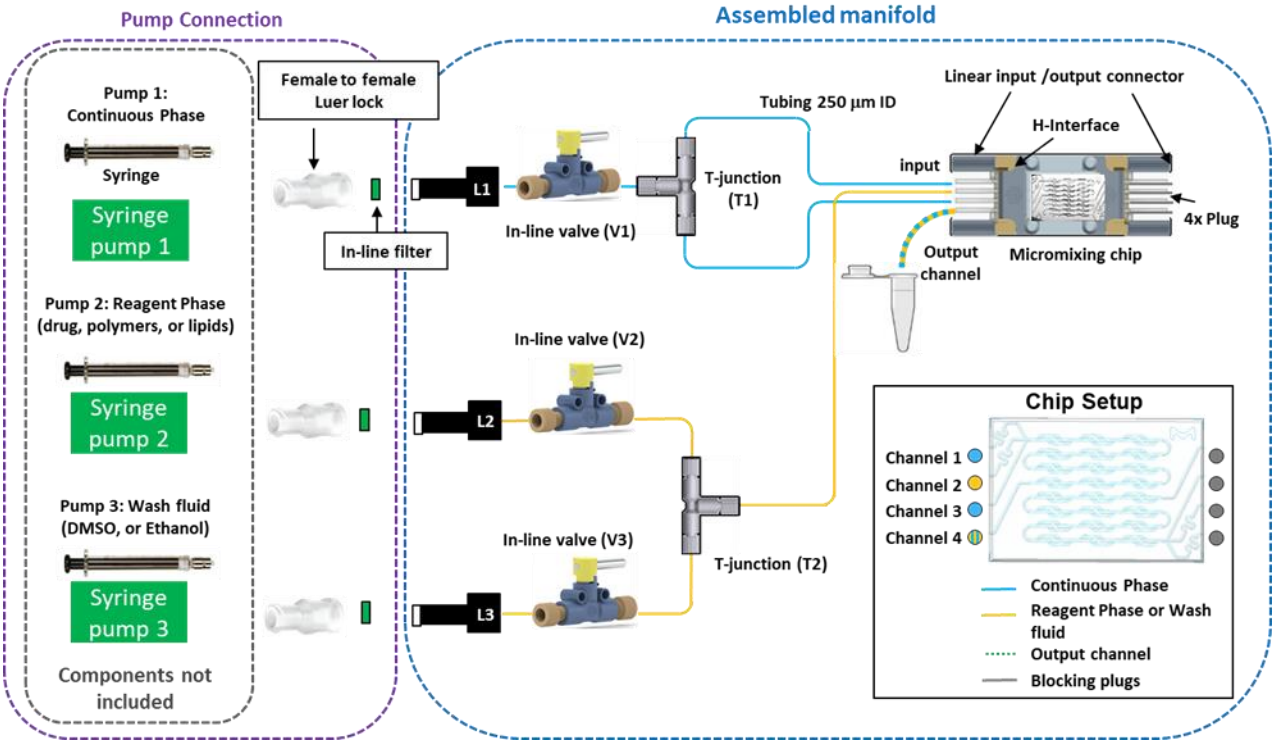

Figure 2: *Schematic of the microfluidics setup for syringe pumps. The manifold is supplied preassembled. The microfluidics chip is packaged separately. In-line filters and luer locks are supplied for connection to syringe pumps.*

A. Assemble the Manifold

- The included kit components are preassembled for ease of use, **Figure 1** and **Figure 2**. The microfluidics chip is packaged separately in a gel-pack case and must be fitted into the manifold as described in section C below.
- We recommend using Mitos P-Pumps from Dolomite Microfluidics (https://www.dolomite-microfluidics.com/product/mitos-p-pump/) (**Figure 1**). Alternatively, the manifold can be connected to syringe pumps using the optional female to female luer lock adaptors provided with the kit (**Figure 2**).
- As shown in the figures, connect the three input lines (L1, L2, L3) to your pumps (pump 1, pump 2, and pump3) respectively.

Note: *The synthesis of nanoparticles using the Dolomite Microfluidics system or syringe pumps can be carried out in either a three-pump (as shown in **Figure 1** and **Figure 2**) or two-pump configuration (pump 1 and 2 only). In the three-pump configuration, a vial of priming solution is kept in pump 3 throughout the process. Pump 3 can be used for the washing step without the need for swapping the vials in pump 2 when using a two-pump configuration. For washing, simply close valve V1 and V2 and open valve V3 and start the flow of solvent using the pump software.*

Note*: Line L1, connected to pump 1, directs flow of the continuous phase (stabilizer or buffer) into channels 1 and 3 of the microfluidics chip via T-connector (T1). Line L2 and L3, connected to pump 2 and 3 respectively, direct flow of reagent phase (drug, polymers or lipids) or wash solution to channel 2 of the microfluidics chip via T-junction connector (T2).*

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- In-line filters are included in the kit and can be assembled in between luer lock and end fittings marked as L1, L2 and L3 when using a syringe pump. If Dolomite Mitos-P pumps are used, in-line filters can be assembled in between the output tube from the pump sensor and input line L1, L2 and L3. Simply place the in-line filter inside the luer lock or output connector and tighten the end fitting.
- In-line valves V1, V2 and V3 are preassembled to control the flow of fluids from Pump 1, 2 and 3 respectively.
- A 15 cm tube is connected to the output channel on the microfluidics chip for collecting the synthesized nanoparticles or waste.

B. Priming the System

Note: *Priming with your solvent is required before making particles. DMSO is recommended as a priming solvent for use with polymers. Alternatively, if the system is going to be used for liposome synthesis, ethanol can also be used as a priming solvent. Priming with a solvent enables wetting of the chip surface and helps to avoid precipitation of reagents inside the system in the case of backflow, jetting or chaotic mixing conditions. The priming procedure also purges gases from the fluid pathways, and conditions the chip surface with your selected solvent or buffer system.*

- Place a clean vial (Cat. No. V7130) containing 10 ml of DMSO or ethanol (filtered using a 0.45 µm syringe filter, Cat. No. SLFH025) inside each pump.
- Loosen the two screws by hand to remove the input connector from the H-interface on the preassembled manifold (**Figure 3A**).
- Set the pressure to 1000 mbar on pump 1 using the Flow Control Center software for the Dolomite microfluidics system, or set the flow rate to 100 µl/min using the syringe pump interface.

Note: *When using syringe pumps, gradually increase flow rate in a stepwise fashion to the desired flow rate. Ensure valves are open in the flow path since back pressure can easily build up and cause leaks.*

- Open valve V1 to start the flow of DMSO or ethanol. Within a minute, droplets of solvent will form on the gasket at channels 1 and 3 (**Figure 3B**).
- Use lint free paper to wipe droplets from the gasket. Monitor for an even rate of droplet formation. (See troubleshooting if uneven droplet formation occurs).
- Close valve V1 and turn off pump 1 using the software or pump interface.
- Repeat the same process with pumps 2 and 3 and observe formation of droplets at channel 2 **(Figure 3C)**.

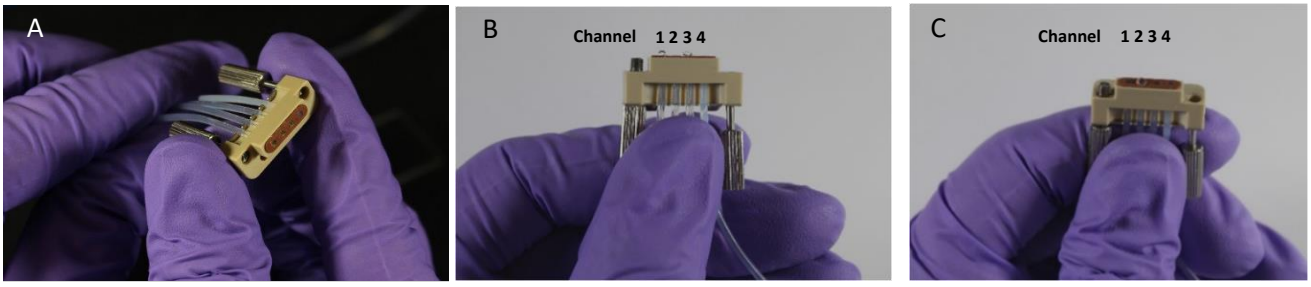

Figure 3: *(A) Input connector from the linear input/output connector, (B) droplets of priming fluid forming on the gasket at channels 1 and 3 and (C) droplet formation at channel 2*

C. *Assemble Microfluidics chip*

- Remove the microfluidics chip from the case and place into the H-interface. Make sure the chip channels are aligned with the tubing on the input connector (**Figure 4A**).
- Connect input and output connectors to the H-interface assembly (**Figure 4A and 5B**).
- First loosely tighten the two screws on each of the input and output connectors, then tighten evenly until finger tight.

  Note: *Ensure the microfluidic chip is aligned with the connectors as shown in* ***Figure 4****. Tighten the interface evenly and securely, while pressing the chip firmly against the H-interface. If chip sits in the interface correctly, the gasket will visibly press against the chip, with the four tubing positions easily identifiable (****Figure 4C****). A poorly connected chip will not have all four tubing positions visible. A poor seal between the chip and gasket will likely cause a leak in the system.*

- Place the assembled chip interface on a flat surface for particle synthesis. If you have a high-speed microscope, place the assembled chip on the stage of a light microscope to observe the flow in the channels during your experiment.

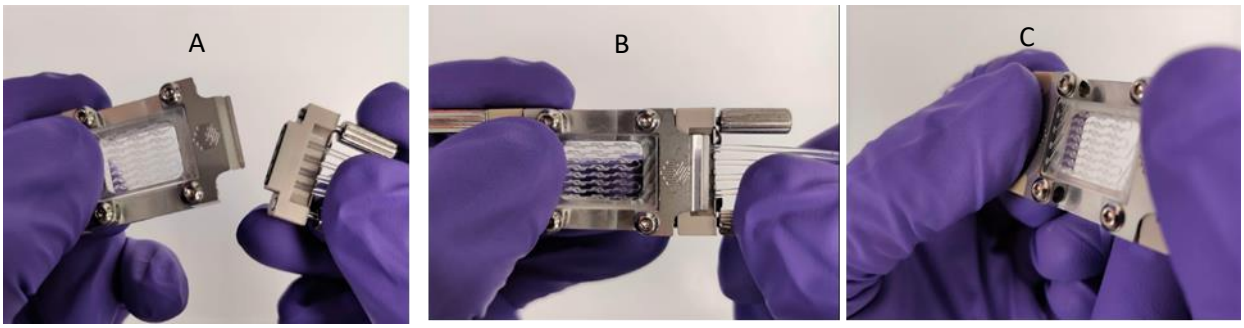

Figure 4: *(A) Align input line from linear input/output connector and H-interface with chip, (not shown) connect output connector with blocking plugs to the other side of the H-interface (B) hand tighten the two screws on each side of the input and output connectors, and (C) check correct alignment between chip and linear input/output connector gasket*

D. *Synthesis of drug-encapsulated nanoparticles or liposomes*

For synthesis of nanoparticles or liposomes, detailed protocols are included with the respective ***NanoFabTx™*** reagent kits (sold separately). Further information is available at www.sigmaaldrich.com/nanofabtx. Below is a general protocol to synthesize nanoparticles or liposomes using the assembled microfluidic device. The flow rates for the continuous phase and reagent phase require optimization to synthesize nanoparticles or liposomes using different polymers, lipids, and drugs.

1. *Prepare microfluidics system*
   - Place a vial (Cat. No. V7130) containing 10 ml of stabilizer solution inside pump 1 and the vial (Cat. No. V7130) of drug/polymer solution inside pump 2.

Note: *For liposome synthesis, place a vial of buffer or water inside pump 1 and the lipid solution in ethanol in pump 2*. For hydrophilic drugs, the drug/buffer solution should go in pump 1; for hydrophobic drugs, the drug/lipid solution should go inside pump 2.

- Check that valves V1, V2, and V3 are closed. Always keep valve V3 closed when using the two-pump configuration.

2. *Optimize flow ratios*
   - The flow rate ratios of the two solutions need to be optimized together to achieve the desired size of nanoparticles or liposomes. This is an iterative process, and particle size should be checked at each flow ratio.
   - Open valve V1 and optimize the flow rates for pump 1. A flow rate between 100-500 µl/min is generally a good starting point.
   - Similarly, optimize the flow rate for pump 2. A flow rate between 50-100 µl/min is generally a good starting point.

3. *Encapsulate drug in nanoparticles or liposomes*
   - Set desired flow rates for each pump based on your optimization using the Flow Control Center software of the Dolomite microfluidics system or on the syringe pump interface.
   - The flow rates of both solutions will stabilize within a few seconds. Optional: You can observe the fluid flow of the two solutions if you have a high-speed microscope.
   - After the flow rates of the two solutions have stabilized, replace the waste collection vial with a sample collection vial at the output channel and collect the drug-encapsulated nanoparticle or liposomes suspension.
   - When you have collected the desired volume of the drug-encapsulated nanoparticle suspension, transfer the output channel tubing to the waste collection vial, close valves V1 and V2 using the Flow Control Center software or syringe pump interface to stop fluid flow, and remove the solution vials from pump 1 and pump 2.
   - Measure size of drug-encapsulated nanoparticles or liposomes with a dynamic light scattering instrument and TEM.
   - Clean the microfluidics system after each use using the method below. Improper cleaning can result in chip and tubing blockages.

E. Clean the microfluidics system

- Follow this cleaning procedure after each run to remove any remaining reagent precipitates or deposited stabilizer.
- Use DMSO to clean the tubing and micromixing microfluidics chip. DMSO is the preferred cleaning solvent, because both the stabilizer and many polymers have high solubility in DMSO. Ethanol is also recommended for use as a cleaning solvent if the device was used with lipids. Alternatively, additional solvents (e.g. DCM, Chloroform) that are compatible with the tubing and can dissolve polymers, lipids, or stabilizer may be used as a cleaning solvent.
- Filter 10 ml DMSO, or other cleaning solvent, through a 0.45 µm syringe filter (Cat. No. SLFH025) into each of three vials (Cat. No.V7130).
- Close Valves V1, V2 and V3, place a waste collection vial at the output channel.
- Place the vials of filtered cleaning solvent in Pumps 1, 2 and 3.
- Open valve V1 and set the flow rate of pump 1 to 100 µl/min.

- Set the flow rate of pump 2 to 100 µl/min and immediately open valve V2.

Note: *If using a three-pumps configuration, washing is not required for pump 3*

- Gradually increase the flow rate on both pumps to 300 µl/min. Run the system for 3 minutes to completely remove any polymer, lipids, or stabilizer precipitated inside tubing or on the micromixing microfluidics chip.
- When the cleaning process is complete, close valves and use the software to immediately stop the flow of the liquids through the pumps.
- Remove the vials that contained cleaning solvent.
- Disconnect the linear input/output connector and remove the micromixing microfluidics chip from the H-interface.
- Store the micromixing microfluidics chip in its box or in a clean, dust-free environment.

Troubleshooting

Due to the numerous connections between microfluidics components, and the narrow flow paths for the fluids, you may encounter leaks or blockages. This section presents information on and potential solutions for commonly encountered problems.

1. Uneven flow in the microfluidics-based method

Possible cause – Uneven flow can be caused by bubbles of air in the system.

Solution – Allow fluid to flow through the system for 1–2 minutes to clear bubbles. You can usually see the bubbles passing through the micromixing microfluidics chip. If this approach does not remove the bubbles, sonicate the solutions for 30 min and vent the pressure chamber.

Possible cause – If the flow becomes unstable when the microfluidics system has been in operation for a while, one of your fluid bottles may have run dry or the pick-up tubing might not reach to the bottom of a vial.

Solution – Check that the vials contain enough reagent and that the 250-µm pick-up tubing is long enough to collect from the bottom of each vial.

Possible cause – If none of the above solutions leads to even flow, restart the syringe pump or software system.

Solution – Stop all flow, close and reopen the Flow Control Centre software, and restart flow. If this method does not solve the problem, the system may have a blockage. Check for blockages as detailed in the next section.

Possible cause – If the system has no blockages, the flow sensor may not function correctly.

Solution – Replace the flow sensor.

2. Leak in system

Possible cause – Changes/fluctuations in system pressure or flow rate can arise from a leak in the system.

Solution – Before troubleshooting a possible blockage, make sure that all connectors are properly fitted and that the system has no apparent leaks.

3. **Blockage of tubing or micromixing microfluidics chip**

Possible cause – During the synthesis of nanoparticles or liposomes using the microfluidics setup, the introduction of dust fibers, deposition of precipitated polymers/lipids/stabilizer, drying of reagents inside the micromixing microfluidics chip or tubing, or improper cleaning procedures can cause blockage in the micromixing microfluidics chip or tubing.
Several indications suggest that a partial or complete blockage has occurred:

a. Consistent flow rate is maintained when a pump is in flow control mode, but the pressure increases.
b. Consistent pressure is maintained when a pump is in pressure control mode, but the flow rate decreases.
c. The instrument software has set changes to the flow rate, but apparent flow rate does not change.
d. The flow is significantly slower than expected.
e. The flow rate fluctuates unexpectedly and affects droplet stability.

Possible cause – If a partial or transitory blockage is present, the pressure may increase gradually, then suddenly drop as the blockage moves along the flow path, and then increase again when the obstruction becomes lodged.

Solution – Blockages can occur anywhere in the flow path of the system; identifying the location of a blockage is a process of elimination.

Start with the micromixing microfluidics chip, because sometimes blockages (dust or hair) are visible under a microscope. If you find a blockage on the chip, monitor it while you vary the pump pressure to try to dislodge it. If a blockage on the chip cannot be cleared, the chip will need to be replaced.

If you see no physical blockage in the micromixing microfluidics chip, disconnect the chip interface and check whether liquid flows from the tubing. If liquid now flows from the disconnected tubing the blockage is likely either in the chip or the connector was improperly seated against the chip. If the system has a T-connector that splits the flow of a solution into two inputs, check that the flow rates through each input are identical. If the flow is asymmetric, a blockage could be somewhere between the T-connector and the chip. First replace the tubing and see if this fixes the problem; if not, replace the T-connector.

If it is not already apparent which line is blocked, vary the flow rate of the solutions one at a time while observing the ends of the tubing. This step will help to identify which line is blocked.

Work your way back through the system, from the chip to the pump, one component at a time, and check for stable flow at each stage. When you find the section that contains the blockage, simply replace it.

The blockage may have occurred because of particulate contamination in your solution(s). Refilter solutions through a 0.45µm syringe filter before use.

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